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Occurrence, Distribution, and Phenotype of Arylsulfatase A Mutations in Patients With Metachromatic Leukodystrophy

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Occurrence, distribution, and phenotype of arylsulfatase A (ASA) mutations were investigated in 27 patients with metachromatic leukodystrophy (MLD) from Central Europe, mainly from Austria (n = 15) and Poland (n = 9). Genomic DNA from leukocytes, fibroblasts, or paraffin-embedded, formalin-fixed brain or nerve tissue, respectively, was tested by natural or mutated primer-modulated PCR restriction, fragment length polymorphism for the eight most common European mutations: R84Q, S96F, 459+1G>A, I179S, A212V, 1204+1G>A, P426L, and 1401del11bp. The overall identification rate of unrelated MLD alleles was the highest, in adult (90%), medium in juvenile (50%), and lowest in late infantile (36%) MLD patients. The two common alleles, 459+1G>A and P426L, together accounted for 42% of all 50 unrelated MLD alleles investigated; I179S was observed in 6 of 50 MLD alleles (12%). Thus, I179S was far more frequent than hitherto thought and appears to be a third common mutation in Europe. Moreover, a different allelic distribution between Austrian and Polish juvenile patients was disclosed, indicating genetic heterogeneity of MLD even within Central Europe. The genotype-phenotype correlation suggested by Polten et al. [N Engl J Med 324:18–22, 1991] was not followed by all of our MLD patients. Moreover, some MLD patients with identical ASA mutations presented with different phenotypes. This may be due, at least in some cases, to the presence of an additional mutation on individual mutant alleles. Therefore, prediction of the clinical course from single mutation analysis is not possible. Am. J. Med. Genet. 69:335–340, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: metachromatic leukodystrophy; arylsulfatase A mutations; genotype-phenotype correlation

INTRODUCTION

Metachromatic leukodystrophy (MLD) is an autosomal recessively inherited disease with an estimated frequency of 1 in 40,000. Three major MLD variants have been characterized: late infantile MLD (onset at 1–2 years, severe form with rapid progression), juvenile MLD (onset between 4–12 years, slower development of the disease), and adult MLD (onset beyond 16 years, usually less severe symptoms and protracted course) [Kolodny and Fluharty, 1995]. There is general agreement on the considerable heterogeneity within these variants with respect to onset and clinical course.

The cause of MLD is the genetic deficiency of the lysosomal enzyme arylsulfatase A (ASA; EC 3.1.6.1). This results in accumulation of the enzyme substrate sulfatide (cerebroside sulfuric ester) in the central and peripheral nervous systems, leading to severe myelin breakdown. Sulfatide accumulates also in visceral organs, e.g., kidney, and is excreted in high amounts in urine.

The ASA gene maps to chromosome 22q, covers 3.2kb of genomic DNA, and includes eight exons [Kreysing et al., 1990]. By now, at least 57 MLD-related ASA mutations have been described [Gieselmann et al., 1994]. Occurrence and frequency of MLD mutations differ within different ethnic groups. Mutations 459+1G>A and P426L, the two most frequent mutations in Europe, each accounting for ~25% of all MLD alleles [Pol-

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ten et al., 1991], could not be detected so far in Japanese patients [Eto et al., 1993]. G99D [Kondo et al., 1991], however, was found exclusively in Japanese patients [Eto et al., 1993].

MLD mutations can be divided functionally into two groups: alleles resulting in enzymatically inactive ASA (0 alleles) and alleles encoding for ASA with residual enzyme activity (R alleles). A recently described genotype-phenotype relation suggests that homozygosity for 0 alleles results in late infantile MLD, whereas presence of two R alleles leads predominantly to adult MLD. Compound heterozygosity for 0/R is most frequent in juvenile MLD. Less frequently, homozygosity for R alleles is also found in the juvenile form [Polten et al., 1991].

In addition to ASA mutations causing MLD, there is also a mutation leading to ASA pseudodeficiency (ASA-PD) [Gieselmann et al., 1989]. This condition of low ASA activity was originally described in clinically healthy individuals [Dubois et al., 1975]. The ASA-PD allele has a relatively high population frequency of 7–15% [Hohenschutz et al., 1989]. It was found that MLD mutations occur also within this allele [Gieselmann et al., 1991; Barth et al., 1993].

In the present study we investigated 27 MLD patients from Central Europe (15 Austrian, 9 Polish, 2 German, and 1 Croatian) to study occurrence, distribution, and genotype-phenotype correlations of 8 European MLD mutations. Mutations investigated were the 0 mutations S96F [Gieselmann et al., 1991], 459+1G>A [Polten et al., 1991], 1204+1G>A [Fluharty et al., 1991], 1401del11bp [Bohne et al., 1993], and the R mutations R84Q [Kappler et al., 1991], I179S [Fluharty et al., 1991], A212V [Barth et al., 1993], P426L [Polten et al., 1991] (Table I). The mutations nomenclature is according to Beaudet and Tsui [1993]. At the time of the experimental design, these were the only known European mutations; since then additional mutations occurring in particular European families have been reported.

MATERIALS AND METHODS

Patients

We investigated a total number of 27 MLD patients (Table II) from Austria, Poland, Germany, and Croatia. Unrelated patients (n = 25) were grouped into the three major MLD phenotypes (7 late infantile, 13 juvenile, and 5 adult MLD patients) according to Kolodny and Fluharty [1995]. This classification is based mainly on age of onset and the clinical course of the disease. However, it takes into account heterogeneity of these parameters within each group. Data from two affected siblings of MLD patient 4 and 25, respectively, are also given in Table II, but were excluded from all statistical evaluations. Diagnosis of MLD was confirmed biochemically by assaying of ASA activity and galactosylsulfatide in urine or brain and by neuropathological examination, respectively (Table II) [Sluga et al., 1975; Pilz et al., 1971, 1977; Kothbauer et al., 1977; Molzer et al., 1992; Minauf et al., 1993]. Normal control values for ASA (nMol p-nitrocatechol/mg protein/hr; mean ± S.D, (range)) were 137.4 ± 53.1 (75–288) in leukocytes and 683 ± 183 (450–948) in fibroblasts; normal control values for sulfatide (nMol/mg lipid; mean ± S.D, (range)) 2.3 ± 1.7 (0–5).

Source and Preparation of DNA

Genomic DNA was prepared from leukocytes or cultured fibroblasts by standard procedures [Sambrook et al., 1989]. If such material was not available, paraffin-embedded, formalin-fixed brain or nerve tissue was used, as described recently [Berger et al., 1993].

Natural PCR Restriction Fragment Length Polymorphism (RFLP)

Mutations S96F, 1204+1G>A, and 1401del11bp each destroy a restriction site; A212V, in contrast, induces one. To detect these mutations, PCR-RFLP assays were performed similar to those described [Fluharty et al., 1991; Barth et al., 1993; Gieselmann et al., 1993]. Mutations, restriction sites, and size of amplified and cleaved fragments in normal and mutant alleles are summarized in Table I.

PCR reactions were done in 50 μl 10 mM Tris/HCl buffer (pH 8.3) containing 0.2 mM dNTP's, 4% DMSO, 1 mM $MgCl_2$, 50 mM KCl, 10 pmol of each primer (Table IIIa,b), and 2.5 units of Taq DNA polymerase (Biomedica), using a thermal cycler (Hybaid). A total of 35 cycles of 1 min denaturation at 94°C, 1 min annealing at the appropriate temperature (Table IIIb), and 1 min elongation at 72°C were performed.

TABLE I. ASA Mutations Investigated

		Characterization of the mutation			Characterization of the assay				
						Cleavage fragments			
Allele	Reference	DNA level	Protein level	Type	Enzyme	Wild type		Mutation	RFLP[d]
R84Q	Kappler et al., 1992	CGG → CAG	Arg 84 → Gln	R	PvuII	100	→	71 + 29	P
S96F[a]	Gieselmann et al., 1991	TCC → TTC	Ser 96 → Phe	0	SmaI	140 + 50	→	190	N
459 + 1G > A[b]	Polten et al., 1991	AGgt → AGat	Splice site	0	XbaI	160	→	130 + 26	P
I179S	Fluharty et al., 1991	ATC → AGC	Ile 179 → Ser	R	PstI	240	→	203 + 33	P
A212V	Barth et al., 1993	GCC → GTC	Ala 212 → Val	R[c]	AatII	222	→	133 + 85	N
1204 + 1G > A	Fluharty et al., 1991	GGgt → GGat	Splice site	0	BstEII	102 + 38	→	145	N
P426L	Polten et al., 1991	CCG → CTG	Pro 426 → Leu	R	PstI	100	→	70 + 26	P
1401del11bp	Bohne et al., 1991	11bp deletion	Frameshift	0	PvuII	194 + 49	→	244	N

[a]On one allele together with ASA-PD.
[b]This allele also bears other nucleotide exchanges probably not related to MLD.
[c]Hypothetical.
[d]N and P denote natural and primer induced RFLPs, respectively.

TABLE II. Clinical, Biochemical, and Genetic Data of the 26 MLD Patients Investigated

#	Orig[a]	Age[b]	Gender[c]	ASA[d]	SULF[e]	DNA[f]	Genotype: MLD mutation	Genotype: Type	Genotype: ASA-PD[g]	Reference
Late infantile type (n = 7)										
1	A	2.5	f	9	↑	P[h]	459 + 1G > A/P426L	O/R	nd	
2	A	2.5	m	nd	↑	P[h]	459 + 1G > A/X	O/X	nd	
3	A	3	f	17	78	L	459 + 1G > A/X	O/X	no/no	
4[i]	A	3.5	m	9	280	P[h]	459 + 1G > A/X	O/X	nd	
5	A	2	f	7	134	L/F	X/X	X/X	no/no	
6	PL	2	m	24	74	L	X/X	X/X	no/no	
7	PL	2	m	9	137	L	X/X	X/X	no/no	
Juvenile type (n = 13)										
8	A	6	f	9	↑	F	459 + 1G > A/P426L	O/R	no/no	
9	A	7	m	0	65	F	459 + 1G > A/P426L	O/R	no/no	
10	A	5	m	5	nd	P[h]	I179S/P426L	R/R	nd	
11	A	10	m	5	86	F	P426L/X	R/X	no/no	
12	A	11	m	8	↑	P[h]	P426L/X	R/X	nd	Sluga et al., 1975
13	PL	10	f	9	nd	L	I179S/X	R/X	PD/no	
14	PL	11	m	14	412	L,F	I179S/X	R/X	no/no	
15	PL	11	f	15	nd	F	I179S/X	R/X	no/no	
16	PL	4	f	25	nd	L	P426L/X	R/X	no/no	
17	PL	4	m	19	154	L	X/X	X/X	PD/no	
18	PL	5	m	13	146	L	X/X	X/X	no/no	
19	PL	6	f	12	nd	L	X/X	X/X	no/no	
20	HR	4	f	38	178	F	459 + 1G > A/X	O/X	no/no	
Adult type (n = 5)										
21	A	29	m	0	280	F	459+1G>A/I179S	O/R	no/no	Minauf et al., 1993
22	A	27	m	6	75	F	P426L/P426L	R/R	no/no	
23	A	29	m	0	127	F	P426L/P426L	R/R	no/no	
24	A	50	m	nd	nd	P[h]	P426L/X	R/X	nd	Kothbauer et al., 1977
25[j]	D	39	f	<6	↑	P[h]	I179S/P426L	R/R	nd	Pilz et al., 1971
Siblings (n = 2)										
26[i]	A	5	m	12	137	L	459 + 1G > A/X	O/X	no/no	
27[j]	D	43	m	↓	↑	P[h]	I179S/P426L	R/R	nd	Pilz et al., 1977

[a]Country of origin: A, Austrian, PL, Poland, HR, Croatia, D, Germany.
[b]At time of laboratory diagnosis.
[c]f = female, m = male.
[d]Arylsulfatase A activity in leukocytes (≠15, 20 in fibroblasts), nmoles .mg protein^{-1}.hour.
[e]Sulfatide excretion in urine (nmoles.mg lipid^{-1}).
[f]Material for DNA preparation: leukocytes (L), fibroblasts (F) or paraffin-embedded, formalin-fixed tissue (P) from sural verve biopsy (12) or brain autopsy.
[g]Arylsulfatase A pseudodeficiency: nd = not done, no = normal.
[h]Not investigated for 1204 + 1G > A.
[i,j]Siblings.
↑ increased, ↓ decreased (semiquantitative assays).

PCR products were precipitated with ethanol, resuspended in restriction enzyme buffer, and digested with the appropriate restriction enzyme for 3 hr at 37°C (BstEII at 60°C). Fragments obtained were separated on 3% agarose gel and visualized by ethidium bromide staining.

Mutated Primer-Modulated PCR Restriction Fragment Length Polymorphism

Mutations R84Q, 459+1G>A, I179S, and P426L do not induce or destroy common restriction sites. Therefore, we introduced restriction sites by PCR amplification using primers matching immediately 5′ or 3′ to the respective mutation. These primers contained one or two mismatches (Table IIIa) such that elongation of these primers created a diagnostic restriction site by "site-directed *in vitro* mutagenesis" in the case of the mutation. This primer modulated PCR-RFLP was described recently for the mutations 459+1G>A and P426L [Berger et al., 1993]. Mutations R84Q and I179S were detected in a similar manner (Table I).

Auxiliary PCR

When MLD mutations were investigated in DNA isolated from paraffin-embedded formalin fixed brain or nerve tissue, an auxiliary PCR amplification preceeded the mutation specific PCR. Primers used for both reactions are compiled in Tables IIIa,b. For auxiliary PCR, 30 cycles of 1 min denaturation at 94°C, 1 min annealing (Table IIIb), and 1 min elongation at 72°C were performed. Five μl of the amplification product were diluted 10-fold in PCR buffer containing the mutation-specific primers (Tables IIIa,b) and subjected to an additional 35 cycles. Ethanol precipitation, restriction cleavage, and detection of remaining fragments were performed as described above.

PCR Controls

Negative controls included DNA from healthy controls as well as reactions containing no template DNA. Positive controls were performed to ensure the complete cleavage of PCR fragments by the restriction enzymes. For mutations S96F, 1204+1G>A, and

TABLE III. PCR Primers

(a) Primers used

#	nt	Sequence (5′ → 3′)[a]
1	331	TCTGTCTGTCTCAGGGACTCTGTGAC
2	430	GGCACCAGGACGCCAGGGTACATGCCCA*G*C
3	389	GCTCCCGGTTCGGATGGGCATGTACCCTGG
4	578	TCGATGGAAGCCCTGATGGGGGGGCAGG
5	579	TTTCTAGGCATCCCGTACTCCCACG*TCT*AG
6	738	GGTTCTGGCAGGGGCCCTGAGGCGGGC
7	533	GGGGCCTGAGGGGGCCTTCC
8	762	CCTTGCGACGGTGGCTGTGACCAGGGCCTGGTC*TG*CA
9	1001	TGGAGTTAGCACTGGGTAGGGGTCAACGGG
10	1090	CAGGGAGTCCCCAAATGGCCCGCGGCC
11	983	GGGGTCAACGGGCAGCCAGGGGGTTG*A*G*A*CAAG
12	2156	GCGGACTGGAAAGTACAAGGCTCAC
13	2300	GGGGCCAATTCTGTGCACAG
14	2311	TCTGCCCACAGTGATACCACT*C*CAGACC
15	2410	CAGGGTCCTTGGACAGGTCAT*A*GAGC*T*GC
16	2461	CTGCAAGCCCTGAAACAGCTTC*G*GCTG*C*TC
17	2704	CCCCCTCCAGACACCTGAGCCT*C*CCCC
18	2723	GTCACAGCTGCAAGTCTCCACTGGTGTAAT

(b) Primers, size of fragments, and annealing temperature used for mutation analysis[b]

	Mutation specific PCR				Auxiliary PCR			
	Primer #		Fragment	Annealing	Primer #		Fragment	Annealing
Mutation	F	R	size	temp (°C)	F	R	size	temp (°C)
R84Q	1	2	100	56	1	4	248	56
S96F	3	4	190	56	1	4	248	56
459 + 1G > A	5	6	160	65	7	6	206	64
I179S	8	9	240	56	8	10	329	56
A212V	8	11	222	56	8	9	240	56
1204 + 1G > A	12	13	145	56	12	15	255	56
P426L	14	15	100	62	12	15	255	56
1401del11bp	16	17	244	72	16	18	292	56

[a]Underlined sequences indicate mismatches compared to the genomic sequence (EMBL data bank accession number X52150).
[b]F, R, forward, and reverse PCR primer. Auxiliary PCR was used before mutation specific PCR only in case of DNA from paraffin-embedded material.

1401del11bp, genomic DNA of healthy controls was amplified and cleaved. To prove appropriate cleavage of mutation A212V by the AatII restriction enzyme, a fragment of the adrenoleukodystrophy gene containing an AatII restriction site of appropriate size was PCR amplified and cleaved. For the mutations R84Q, 459+1G>A, I179S, and P426L, plasmids containing the ASA gene with the appropriate mutations were constructed; 100–1,000 copies of each plasmid were used as positive control DNA template. Each type of control experiments was performed simultaneously with the patients samples.

Hybridization With Mutation-Specific Oligonucleotides

DNA of patient #22 was investigated by hybridization with mutation-specific oligonucleotides as described by Polten et al. [1991] for P426L. Patients 11, 21, and 23 were investigated by hybridization according to Polten et al. [1991] (459+1G>A, P426L), or Fluharty et al. [1991] (I179S), respectively, as well as by the mutation specific PCR assays.

ASA Pseudodeficiency Allele

The ASA-PD allele was investigated as described by Gieselmann [1991].

RESULTS

ASA Alleles Detected

Preliminary experiments demonstrated that the mutations R84Q, S96F, 459+1G>A, I179S, A212V, 1204+1G>A, P426L, and 1401del11bp were detected correctly by the appropriate assay, using either plasmids encoding the respective allele or genomic DNA with confirmed sequences as templates. The specificity of these assays was substantiated by the results from the PCR control experiments.

Mutations R84Q, S96F, 459+1G>A, I179S, A212V, P426L, and 1401del11bp were tested on all 27 patients. In addition, 19 patients were investigated for 1204+1G>A and ASA pseudodeficiency. The results are compiled in Table II. Excluding siblings 26 and 27, of the 50 unrelated chromosomes investigated, 27 were identified as carrying mutations. The identification rate was the highest in adult (90%), medium in juvenile (50%), and the lowest in late infantile cases (36%).

Of all MLD mutations tested, only three could be detected: the 0 allele 459+1G>A (n = 8), and the R alleles I179S (n = 6) and P426L (n = 13): 459+1G>A as well as P426L was found in late infantile, juvenile, and adult MLD; I179S was detected only in juvenile and adult patients; P426L was the most frequent mutation in the adult patients (60%).

Genotype-Phenotype Correlations

Complete ASA genotypes (both mutant alleles) were identified in 8 patients (1/7 late infantiles, 3/13 juveniles, and 4/5 adults). One mutant allele was identified in 11 patients (3/7 late infantiles, 7/13 juveniles, and 1/5 adults), and none in 6 patients (3/7 late infantiles and 3/13 juveniles).

In late infantile patients, the complete genotype could be identified in only one case (#1) only. Patient (#1) presented with 459+1G>A and P426L, a 0 and R allele according to Polten at al. [1991]. Three cases carried the 0 mutation 459+1G>A with a still unspecified second allele. Remarkably, one of these patients has a dizygotic twin brother displaying the juvenile phenotype. In the other late infantile patients neither allele was identified.

In juvenile patients, the mutations 459+1G>A, I179S, and P426L were detected. Two juveniles were compound heterozygotes for 459+1G>A and P426L, a 0 and a R allele; this is in concordance with the phenotypic expression proposed by Polten at al. [1991] for 0/R compound heterozygotes. However, another patient (#10) exhibited two R-alleles (I179S/P426L) and showed a faster progress of the disease (death at age of 8 years) than to be expected from his genotype.

The other juveniles presented combinations of either the 0 mutation 459+1G>A (2 patients) or of an R-allele (I179S, 3 patients; P426L, 1 patient) with a presumed unknown mutation, or showed two undetermined alleles (3 patients).

Within the adult group, two patients were homozygous for the R allele P426L, and two siblings were compound heterozygotes for I179S and P426L, all in accordance with the proposed genotype-phenotype correlation [Polten et al., 1991]. However, we also found a 0/R genotype (459+1G>A/I179S) in one adult patient (#22).

Different Distribution of Mutations in Austrian and Polish Patients

In Austrian juveniles, the allele identification rate was 8/10 alleles (80%) and thus markedly higher than in juveniles from Poland (4/14 alleles, 29%; $P < 0.05$; two-sided Chi-square) (Fig. 1). Moreover, 459+1G>A was found only in Austrian cases and P426L occurred more frequently in this group than in Polish patients. In contrast, I179S was detected in 3/7 Polish and only in 1/6 Austrian patients.

ASA Pseudodeficiency

The ASA-PD allele [Gieselmann et al., 1989] was detected in only two juvenile Polish patients (#13 and #17, Table II). Family studies in patient #13 revealed that the I179S mutation was not on the ASA-PD allele. Co-segregation of the PD allele with the MLD mutation S96F [Gieselmann et al., 1991] was not encountered in our patients.

DISCUSSION

Of the eight different ASA mutations tested in our MLD patients of Central European origin, only three could be detected. In addition to the two common mutations 459+1G>A and P426L, I179S was found in 6 of 25 unrelated MLD patients. This frequency of I179S is higher than expected from the data of Fluharty et al. [1991], who found this mutation in 4 of approximately 100 MLD patients. Therefore, I179S seems to be a third mutation with a relatively high incidence in Central Europe. However, mutations R84Q, S96F, A212V, 1204+1G>A, and 1401del11bp could not be detected in any of our patients. This observation, and the fact that in our collective nearly 50% of the MLD alleles remained unidentified, indicates a marked allele heterogeneity in this disease and also the occurrence of other mutations than those investigated. The unidentified mutations may be rare ones characterized before and/or novel mutations. This view is supported by the increasing number of ASA mutations reported to occur only in single or in few patients.

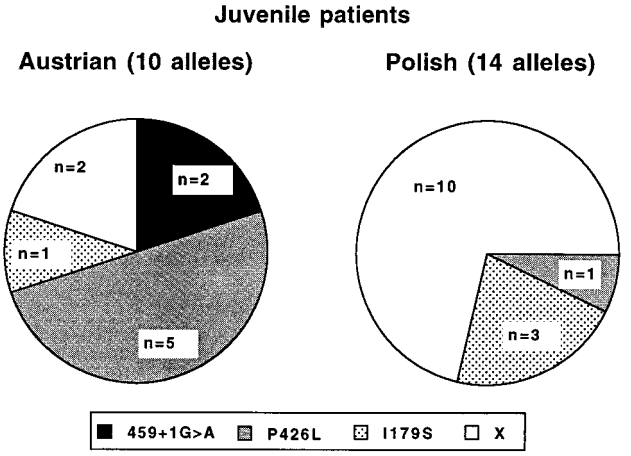

Fig. 1. Allele distribution in Austrian and Polish juvenile patients. The number (n) of alleles carrying the specific mutation is indicated within the appropriate segment. X denotes unidentified alleles.

ASA mutations are known to differ between Caucasian and Japanese patients, but were thought to have a similar distribution within Europe [Eto et al., 1993; Kolodny and Fluharty, 1995]. In this study, we disclosed a difference within a Central European collective. In Austrian juveniles the identification rate was markedly higher than in Polish juveniles. Moreover, in all Austrian patients the frequent mutations 459+1G>A and/or P426L were present, whereas the P426L allele occurred only in one Polish patient. In contrast, I179S was a little more frequent in the Polish group. To our knowledge, neither the Austrian nor the Polish patients belong to particular ethnic groups. Thus our data point to a different allelic distribution of MLD mutations within Central Europe.

The genotype-phenotype correlation suggested by Polten et al. [1991] (0/0, late infantile MLD; 0/R, juvenile MLD; R/R adult MLD) was observed clearly in two juvenile and three adult patients. Three other patients, however, did not follow this pattern. A late infantile patient (#1), deceased at the age of 2, presented with 459+1G>A/P426L (0/R) genotype from which a later onset of disease would have been expected. A similar constellation was observed in a juvenile patient (#10), deceased at the age of 8, presenting with I179S/P426L (R/R) genotype from which adult type MLD would have been predicted. It is conceivable that the R alleles in

both cases might have carryed an additional MLD-related mutation, transforming the R type into a 0 type allele. The occurrence of two disease-related mutations within the same allele was described recently by Kappler et al. [1994] in a MLD patient carrying two deleterious mutations in each of the ASA alleles. In another patient, Barth et al. [1993] suggested that the P426L allele might bear in addition a second, deleterious mutation. This also could have been the case in our patients. However, searching for additional mutations by sequence analysis was not feasible in both cases due to bad preservation of genomic DNA in paraffin embedded brain material [Kösel and Graebner, 1994]. Since two disease-related mutations might occur within the same allele, prediction of the clinical course of a MLD patient from single mutation analysis is not possible.

In contrast, an adult patient (#21; also described earlier) [Fluharty et al., 1991] presented with 459+1G>A/I179S, an 0/R genotype (expected: R/R). I179S was described as an R allele with about 5% of residual ASA activity when expressed in BHK cells. In human 459+1G>A/I179S compound heterozygotes, I179S seems to mitigate MLD, resulting in an adult phenotype [Fluharty et al., 1991]. It might be hypothesized that I179S expressed in human myelinating cells leads to higher residual ASA activity than in the BHK cell system.

A different genetic background irrespective of the gene mutation might be responsible for phenotypic variability, as discussed by McInnes et al. [1992] for Sandhoff disease. However, the degree of impairment of the arylsulfatase A in vivo activity seems to be the most important factor for the clinical presentation of MLD. Future analyses of patients with unexpected genotype-phenotype correlations will distinguish between arylsulfatase A gene-dependent influences (additional mutations within the arylsulfatase A gene) or indirect influences (e.g., Saposin B polymorphism) on the arylsulfatase A in vivo activity and will increase our understanding of genotype-phenotype correlations in MLD.

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